Effect of food intake on biomarkers for cardiovascular disease and inflammation analyzed with the Proseek Multiplex CVD II kit

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ABSTRACT

Objectives: The present study evaluates the effect of food intake on 90 biomarkers for cardiovascular disease (CVD) and inflammation with the Proseek Multiplex CVD II kit.

Methods: Twenty-two healthy subjects (11 male and 11 females aged 25.9 ± 4.2 years) were investigated. A total of 90 biomarkers were measured before a standardized meal, 30 and 120 minutes thereafter with the Proseek Multiplex CVD II kit.

Results: The levels for 27 biomarkers changed significantly after food intake. Two biomarkers increased 120 minutes after food intake, five biomarkers decreased 30 minutes after food intake, seven biomarkers decreased 120 minutes after food intake, and 13 biomarkers decreased both 30 and 120 minutes after food intake. Fourteen biomarkers changed 10% or more after food intake, all 120 minutes after food intake: Heat shock 27 kDa protein (10%), Proto-oncogene tyrosine-protein kinase Src (13%), Growth hormone (13%), Carbonic anhydrase 5A, mitochondrial (14%), Carcinoembryonic antigen related cell adhesion molecule 8 (15%), Fatty acid-binding protein, intestinal (16%), Pentraxin-related protein PTX3 (17%), Fibroblast growth factor 21 (18%), C-C motif chemokine 3 (25%), 2,4-dienoyl-CoA reductase, mitochondrial (28%),

Gastrotropin (36%), Poly [ADP-ribose] polymerase 1 (37%), Interleukin-6 (42%), and Melusin (52%).

Conclusion: The present study shows that food intake affects several different biomarkers analyzed with the Proseek Multiplex CVD II kit, and the effect is at times substantial. Timing of blood sampling in relation to food intake, therefore, appears to be a major concern. Further studies are warranted in older healthy subjects and in patients with various cardiac diseases to determine whether the findings are reproducible.

Key words: Proseek Multiplex CVD II; Olink

INTRODUCTION

Cardiovascular diseases (CVD) are leading causes for both morbidity and mortality and represent a significant burden for the healthcare system worldwide (Cooney et al., 2009). Further improvements of current risk assessment systems for CVD are needed, given the numerous preventive and therapeutic options that are available, including different novel interventional therapies (Cooney et al., 2009; Allan et al., 2014; Zhang et al., 2016; Li et al., 2017a; Li et al., 2017b; Chen et al., 2018). This has resulted in a quest for novel biomarkers that could improve risk assessment for different CVD (Edsfeldt et al., 2016; Goncalves et al., 2015; Gonçalves et al., 2016; Lind et al., 2015a; Lind et al., 2015b; Wiklund et al., 2016; Schiopu et al., 2016). However, digestion of food is known to have significant hemodynamic and metabolic effects (Hlebowicz et al., 2011a; Hlebowicz et al., 2011b; Gårdinger et al., 2014; Dieden et al., 2016; Dencker et al., 2011; Quatela et al., 2016), and may also affect different biomarkers. It is relevant from a practical point of view to investigate whether these biomarkers are affected by food intake, as the timing of blood sampling in relation to food intake would then be relevant. We have previously reported the effects of food intake on biomarkers analyzed with the Proseek Multiplex CVD III and Proseek Multiplex Neurology I kits (Dencker M et al., 2017a; Dencker M et al., 2017b), and now extend our work with the Proseek Multiplex CVD II kit. The present study, therefore, evaluates the effect of food intake in healthy volunteers on 92 different emerging biomarkers for CVD and inflammation analysed with the Proseek Multiplex CVD II kit. To the best our knowledge, this has not been done before.

MATERIAL AND METHODS

The trial is registered at the US National Library of Medicine with the trial registration number NCT01027507. All subjects gave their written informed consent. The study was approved by the regional ethical review board in Lund, Sweden. The study investigated 22 healthy Caucasians (11 male and 11 females, aged 25.9 ± 4.2 years). The subjects were examined between 7.30 and 11.00 a.m. after an 8-h fast. The study populations height and weight were measured and BMI and body surface area (Du Bois D and Du Bois, 1916) were calculated. None of the subjects had a prior history or showed any symptoms of cardiovascular disease or any other chronic disease. None of the subjects were taking any cardiovascular medication. The subjects ingested a standardized meal consisting of 300 g rice pudding (AXA Goda Gröten Risgrynsgröt; Lantmännen AXA, Järna, Sweden). The total caloric value of the meal was 330 kcal: 10% from protein (9 g), 58% from carbohydrates (48 g), and 32% from fat (12 g).

Blood samples

Blood samples were collected before the meal as well as 30 and 120 minutes afterwards and then frozen. No beverages were consumed during the experiment. One of the blood samples collected 30 minutes after the meal was defective and excluded from the analysis. The 92 biomarkers were analyzed by the Proximity Extension Assay technique using the Proseek Multiplex CVD II 96 × 96 reagents kit (Olink Bioscience, Uppsala, Sweden)

as previously described (Lundberg et al., 2011; Assarsson et al., 2014). Data are presented as arbitrary units (AU). Values can be transformed to actual concentrations using transformation algorithms on the Olink Bioscience website (www.olink.com). The conversion, however, is not exact. Most of measurements for Natriuretic Peptides B (97%) failed to reach the detection level and 40% of the samples for Adrenomedullin failed to reach the detection level. These biomarkers were omitted from the analysis. Two samples for Melusin failed to reach detection levels. In these cases, the values were set at the detection levels. The remaining 90 biomarkers that were analyzed were (intra- and inter-assay variation): Bone morphogenetic protein 6 (21%,15%), Angiopoietin-1 (9%,9%), Adrenomedullin (13%, 11%), CD40 ligand (9%,14%), SLAM family member 7 (11%,9%), Placenta growth factor (12%,13%), A disintegrant and metalloproteinase with thrombospondin motifs 13 (4%,10%), Brother of CDO (10%,14%), Interleukin-4 receptor subunit alpha (9%,15%), Proto-oncogene tyrosine-protein kinase Src (10%,12%), Interleukin-1 receptor antagonist protein (12%,36%), Interleukin-6 (9%,9%), Tumor necrosis factor receptor superfamily member 10A (11%,11%), Serine/threonine-protein kinase 4 (7%,10%), Alpha-L-iduronidase (6%,18%), Tumor necrosis factor receptor superfamily member 11A (10%,13%), Proteinase-activated receptor 1 (9%,12%), TNF-related apoptosis-inducing ligand receptor 2 (10%,12%), Serine protease 27 (9%,13%), Angiopoietin-1 receptor (8%,14%), Tissue factor (8%,13%), Interleukin-1 receptor-like 2 (10%,14%), Platelet-derived growth factor subunit B (11%,12%), Interleukin-27 (7%,11%), Interleukin-17D (13%,12%), C-X-C motif chemokine 1 (10%,13%), Lectin-like oxidized LDL receptor 1 (9%,11%), Galectin-9 (5%,13%), Gastric intrinsic factor (11%,15%), Stem cell factor (7%,12%), Interleukin-18 (11%,11%), Fibroblast growth factor 21 (12%,14%), Polymeric immunoglobulin receptor (3%,14%), Receptor for advanced glycosylation end products (9%,11%), Superoxide dismutase [Mn], mitochondrial (6%,9%), Chymotrypsin C (10%,10%), Fibroblast growth factor 23 (14%,15%), Spondin-2 (5%,11%), Growth hormone (7%,9%), Follistatin (9%,15%), Lactoylglutathione lyase (8%,11%), SLAM family member 5 (9%,12%), Pappalysin-1 (13%,12%), Serpin A12 (10%,22%), Renin (8%,12%), 2,4-dienoyl-CoA reductase, mitochondrial (15%,15%), Tyrosine-protein kinase Mer (10%,10%), Kidney injury molecule 1 (11%,9%), Thrombospondin-2 (5%,8%), Thrombomodulin (11%,10%), V-set and immunoglobulin domain-containing protein 2 (8%,10%), Protein AMBP (6%,7%), Prolargin (7%,8%), Heme oxygenase 1 (8%,10%), Lymphotactin (10%,10%), Pro-interleukin-16 (11%,12%), Sortilin (8%,12%), Carcinoembryonic antigen related cell adhesion molecule 8 (11%,10%), Pentraxin-related protein PTX3 (8%,10%), P-selectin glycoprotein ligand 1 (6%,10%), C-C motif chemokine 17 (12%,13%), C-C motif chemokine 3 (9%,8%), Matrix metalloproteinase-7 (9%,9%), Low affinity immunoglobulin gamma Fc region receptor II-b (9%,15%), Melusin (11%,11%), Decorin (7%,11%), Dickkopf-related protein 1 (11%,9%), Lipoprotein lipase (7%,8%), Prostasin (8%,11%), Agouti-related protein (9%,12%), Proheparin-binding EGF-like growth factor (8%,10%), Growth/differentiation factor 2 (9%,11%), Fatty acid-binding protein, intestinal (8%,9%), Thrombopoietin (9%,11%), Macrophage receptor MARCO (6%,9%), Gastrotropin (16%,15%), Natriuretic peptides B (not available), Matrix metalloproteinase-12 (11%,10%), Angiotensin-converting enzyme 2 (8%,11%), Programmed cell death 1 ligand 2 (9%,10%), Cathepsin L1 (10%,10%), Osteoclast-associated immunoglobulin-like receptor (5%,10%), Tumor necrosis factor receptor superfamily member 13B (10%,10%), Protein-glutamine gamma-glutamyl transferase 2 (8%,12%), Leptin (6%,10%), Carbonic anhydrase 5A, mitochondrial (9%,10%), Heat shock 27 kDa protein (11%,12%), T-cell surface glycoprotein CD4 (10%,9%), NF-kappa-B essential modulator (9%,9%), Vascular endothelial growth factor D (7%,10%), Poly [ADP-ribose] polymerase 1 (9%,11%), and Hydroxy acid oxidase 1 (9%,9%).

Statistical analysis

Data are presented as mean ± standard deviation (SD), and the difference ± SD (for the difference) at 30 and 120 minutes after food intake. Statistical analyses were carried out using Statistica 12 (TIBCO Software Inc., Palo Alto, CA, USA). Comparison between fasting values versus 30 and 120 minutes after food intake for any given biomarker was analyzed for significance with repeated measures ANOVA with post hoc analysis by Tukey's test. Statistical significance was set at $P<0.05$.

RESULTS

Table 1 displays descriptive statistics of the study population. The levels for 27 biomarkers changed significantly after food intake. The changes were modest in about half of the cases. The levels of 14 biomarkers changed 10% or more after food intake, all 120 minutes after food intake. Two biomarkers increased 120 minutes after food intake: Lipoprotein lipase (3%) and Interleukin-6 (42%). Five biomarkers decreased 30 minutes after food intake: P-selectin glycoprotein ligand 1 (1%), Thrombospondin-2 (1%), Prolargin (2%), Decorin (2%), and Pappalysin-1 (6%). Seven biomarkers decreased 120 minutes after food intake: C-X-C motif chemokine 1 (2%), Lectin-like oxidized LDL receptor 1 (4%), Heat shock 27 kDa protein (10%), Carbonic anhydrase 5A, mitochondrial (14%), Fatty acid-binding protein, intestinal (16%), Gastrotropin (36%), and Melusin (52%). Thirteen biomarkers decreased both 30 min and 120 min after food intake include: Sortilin (2% and 2%), Leptin (3% and 3%), SLAM family member 5 (3% and 4%), Proteinase-activated receptor 1 (6% and 6%), CD40 ligand (6% and 7%), Fibroblast growth factor 21 (6% and 18%), Poly [ADP-ribose] polymerase 1 (6% and 37%), Proto-oncogene tyrosine-protein kinase Src (7% and 13%), Carcinoembryonic antigen related cell adhesion molecule 8 (8% and 15%), Growth hormone (9% and 13%), Pentraxin-related protein PTX3 (12% and 17%), 2,4-dienoyl-CoA reductase, mitochondrial (14% and 28%), and C-C motif chemokine 3 (24% and 25%). A summary of the findings is displayed in Table 2. Statistical significance is reported at three different levels; *(P<0.05), **(P<0.01), and ***(P<0.001). The values between P<0.05 and P<0.01 should be treated with caution because of the possibility of mass significance.

Table 1. Subjects' anthropometric characteristics. Values are mean ± SD.

Variables	
Sex (male/female)	11/11
Body mass (kg)	69 ± 10
Height (cm)	177 ± 8
BMI (kg/m^2)	21.8 ± 2.2
BSA (m^2)	1.8 ± 0.2

Abbreviations: Body Mass Index (BMI). Body Surface Area (BSA).

Table 2. Summary of findings for 90 biomarkers before, and the differences 30 and 120 minutes after a standardized meal. All values are in arbitrary units (Mean ± SD). *Indicates significant difference (P<0.05), ** (P<0.01), and ***(P<0.001), compared to fasting values.

Variables	Fasting (n=22)	Difference 30 minutes after food intake (n=21)	Difference 120 minutes after food intake (n=22)
Bone morphogenetic protein 6	1.76 ± 0.60	0.01 ± 0.29	-0.02 ± 0.27
Angiopoietin-1	10.36 ± 0.18	-0.04 ± 0.12	0.01 ± 0.19
CD40 ligand	8.29 ± 0.69	-0.47 ± 0.44**	-0.60 ± 0.75***
SLAM family member 7	1.58 ± 0.40	-0.14 ± 0.23	-0.04 ± 0.50
Placenta growth factor	6.94 ± 0.38	-0.10 ± 0.22	0.01 ± 0.58
A disintegrin and metalloproteinase with thrombospondin motifs 13	6.07 ± 0.11	-0.04 ± 0.09	0.03 ± 0.11
Brother of CDO	4.71 ± 0.39	-0.11 ± 0.18	-0.08 ± 0.41
Interleukin-4 receptor subunit alpha	2.33 ± 0.35	-0.16 ± 0.21	-0.16 ± 0.53
Proto-oncogene tyrosine-protein kinase Src	6.06 ± 0.46	-0.45 ± 0.39*	-0.79 ± 0.80***
Interleukin-1 receptor antagonist protein	4.25 ± 0.50	-0.12 ± 0.25	-0.14 ± 0.42
Interleukin-6	1.60 ± 1.10	-0.05 ± 0.29	0.67 ± 0.90***
Tumor necrosis factor receptor superfamily member 10A	2.49 ± 0.39	-0.13 ± 0.19	-0.01 ± 0.47
Serine/threonine-protein kinase 4	1.18 ± 0.48	-0.03 ± 0.44	-0.32 ± 0.57*
Alpha-L-iduronidase	3.48 ± 0.46	-0.13 ± 0.22	-0.03 ± 0.50
Tumor necrosis factor receptor superfamily member 11A	4.88 ± 0.43	-0.17 ± 0.21	-0.13 ± 0.58
Proteinase-activated receptor 1	3.23 ± 0.24	-0.18 ± 0.19*	-0.20 ± 0.33**

TNF-related apoptosis-inducing ligand receptor 2	4.30 ± 0.32	-0.09 ± 0.18	-0.04 ± 0.45
Serine protease 27	8.25 ± 0.36	-0.10 ± 0.19	-0.01 ± 0.40
Angiopoietin-1 receptor	8.56 ± 0.28	-0.02 ± 0.14	0.04 ± 0.33
Tissue factor	4.74 ± 0.41	0.01 ± 0.22	0.17 ± 0.47
Interleukin-1 receptor-like 2	3.55 ± 0.66	-0.05 ± 0.19	0.05 ± 0.47
Platelet-derived growth factor subunit B	10.31 ± 0.16	-0.03 ± 0.13	0.06 ± 0.15
Interleukin-27	3.18 ± 0.26	-0.04 ± 0.12	-0.01 ± 0.15
Interleukin-17D	1.22 ± 0.25	-0.05 ± 0.21	-0.00 ± 0.32
C-X-C motif chemokine 1	8.69 ± 0.44	-0.17 ± 0.22	-0.19 ± 0.39*
Lectin-like oxidized LDL receptor 1	8.23 ± 0.55	-0.18 ± 0.23	-0.29 ± 0.36***
Galectin-9	6.33 ± 0.37	-0.03 ± 0.15	-0.11 ± 0.29
Gastric intrinsic factor	5.20 ± 0.71	0.09 ± 0.17	0.11 ± 0.51
Stem cell factor	9.62 ± 0.36	-0.03 ± 0.13	0.03 ± 0.19
Interleukin-18	7.94 ± 0.54	-0.10 ± 0.23	-0.10 ± 0.59
Fibroblast growth factor 21	4.80 ± 1.08	-0.31 ± 0.31**	-0.86 ± 0.55***
Polymeric immunoglobulin receptor	5.24 ± 0.12	-0.04 ± 0.11	-0.00 ± 0.11
Receptor for advanced glycosylation end products	5.08 ± 0.32	-0.06 ± 0.16	0.00 ± 0.30
Superoxide dismutase [Mn]. mitochondrial	8.18 ± 0.24	-0.07 ± 0.12	-0.01 ± 0.27
Chymotrypsin C	10.10 ± 0.77	-0.04 ± 0.22	0.10 ± 0.48
Fibroblast growth factor 23	1.67 ± 0.34	-0.14 ± 0.22	-0.11 ± 0.38
Spondin-2	8.73 ± 0.15	-0.06 ± 0.11	-0.01 ± 0.19
Growth hormone	8.64 ± 2.61	-0.78 ± 1.37*	-1.11 ± 1.67**
Follistatin	10.88 ± 0.84	-0.09 ± 0.25	-0.13 ± 0.52
Lactoylglutathione lyase	7.41 ± 0.84	0.05 ± 0.88	-0.19 ± 0.99
SLAM family member 5	6.19 ± 0.32	-0.21 ± 0.21**	-0.22 ± 0.34**
Pappalysin-1	2.54 ± 0.62	-0.15 ± 0.23*	0.00 ± 0.34
Serpin A12	3.56 ± 1.74	-0.08 ± 0.34	-0.24 ± 0.48
Renin	6.63 ± 0.49	-0.13 ± 0.16	-0.08 ± 0.44
2.4-dienoyl-CoA reductase. mitochondrial	3.20 ± 0.37	-0.44 ± 0.39**	-0.89 ± 0.53***
Tyrosine-protein kinase Mer	3.51 ± 0.42	-0.08 ± 0.24	-0.01 ± 0.57
Kidney injury molecule 1	6.95 ± 0.48	-0.08 ± 0.19	0.05 ± 0.52
Thrombospondin-2	5.48 ± 0.19	-0.06 ± 0.09**	-0.03 ± 0.07
Thrombomodulin	8.33 ± 0.45	-0.16 ± 0.25	-0.09 ± 0.60
V-set and immunoglobulin domain-containing protein 2	2.78 ± 0.44	-0.12 ± 0.21	-0.20 ± 0.54
Protein AMBP	6.47 ± 0.29	-0.05 ± 0.11	-0.02 ± 0.13
Prolargin	5.72 ± 0.21	-0.11 ± 0.13*	-0.09 ± 0.23
Heme oxygenase 1	9.84 ± 0.50	-0.04 ± 0.16	0.01 ± 0.34
Lymphotactin	4.35 ± 0.62	-0.11 ± 0.17	-0.11 ± 0.42
Pro-interleukin-16	4.88 ± 0.51	-0.15 ± 0.30	-0.22 ± 0.51
Sortilin	6.76 ± 0.22	-0.16 ± 0.15**	-0.13 ± 0.29*
Carcinoembryonic antigen related cell adhesion molecule 8	6.17 ± 0.72	-0.50 ± 0.62*	-0.95 ± 0.87***
Pentraxin-related protein PTX3	2.42 ± 0.61	-0.28 ± 0.27*	-0.41 ± 0.49***
P-selectin glycoprotein ligand 1	3.92 ± 0.15	-0.05 ± 0.10*	-0.04 ± 0.07
C-C motif chemokine 17	8.31 ± 0.95	-0.18 ± 0.27	-0.05 ± 0.51
C-C motif chemokine 3	2.47 ± 1.24	-0.60 ± 0.83**	-0.61 ± 1.02**
Matrix metalloproteinase-7	9.64 ± 0.44	-0.13 ± 0.23	-0.09 ± 0.52
Low affinity immunoglobulin gamma Fc region receptor II-b	1.46 ± 0.43	-0.01 ± 0.17	-0.02 ± 0.18
Melusin	1.03 ± 0.58	-0.21 ± 0.44	-0.54 ± 0.59***
Decorin	4.55 ± 0.24	-0.10 ± 0.12*	-0.05 ± 0.18
Dickkopf-related protein 1	10.17 ± 0.24	-0.10 ± 0.13	-0.03 ± 0.29
Lipoprotein lipase	8.47 ± 0.58	-0.11 ± 0.20	0.24 ± 0.38***
Prostasin	8.35 ± 0.32	-0.07 ± 0.17	-0.02 ± 0.40
Agouti-related protein	2.28 ± 0.47	-0.10 ± 0.21	-0.09 ± 0.48
Proheparin-binding EGF-like growth factor	5.95 ± 0.68	-0.32 ± 0.52	0.02 ± 0.75
Growth/differentiation factor 2	4.65 ± 0.46	-0.06 ± 0.23	0.08 ± 0.39
Fatty acid-binding protein, intestinal	8.37 ± 0.76	-0.28 ± 0.39	-1.36 ± 0.71***
Thrombopoietin	2.45 ± 0.40	-0.14 ± 0.20	-0.07 ± 0.48
Macrophage receptor MARCO	5.97 ± 0.30	-0.08 ± 0.17	-0.03 ± 0.30
Gastrotropin	1.10 ± 0.45	-0.09 ± 0.21	-0.40 ± 0.38***
Matrix metalloproteinase-12	5.58 ± 0.62	-0.12 ± 0.25	-0.07 ± 0.65
Angiotensin-converting enzyme 2	2.60 ± 0.44	-0.11 ± 0.17	-0.03 ± 0.34
Programmed cell death 1 ligand 2	2.48 ± 0.43	-0.13 ± 0.17	0.02 ± 0.46
Cathepsin L1	5.01 ± 0.32	-0.05 ± 0.20	-0.03 ± 0.30
Osteoclast-associated immunoglobulin-like receptor	9.51 ± 0.28	-0.05 ± 0.14	-0.01 ± 0.23
Tumor necrosis factor receptor superfamily member 13B	7.45 ± 0.45	-0.10 ± 0.17	-0.04 ± 0.41
Protein-glutamine gamma-glutamyltransferase 2	6.05 ± 0.96	0.26 ± 0.95	-0.14 ± 1.02
Leptin	5.21 ± 1.52	-0.15 ± 0.15*	-0.15 ± 0.34*
Carbonic anhydrase 5A. mitochondrial	1.18 ± 0.64	-0.11 ± 0.18	-0.16 ± 0.34*
Heat shock 27 kDa protein	9.46 ± 0.94	-0.41 ± 0.69	-0.95 ± 0.86*
T-cell surface glycoprotein CD4	3.48 ± 0.33	-0.14 ± 0.22	-0.09 ± 0.40
NF-kappa-B essential modulator	4.77 ± 0.49	-0.13 ± 0.49	-0.34 ± 0.66
Vascular endothelial growth factor D	6.76 ± 0.41	-0.07 ± 0.14	0.03 ± 0.29
Poly [ADP-ribose] polymerase 1	0.99 ± 0.34	-0.06 ± 0.44*	-0.37 ± 0.57**
Hydroxyacid oxidase 1	3.10 ± 1.24	-0.11 ± 0.22	-0.09 ± 0.49

DISCUSSION

To our knowledge, this is the first study to evaluate the effect of food intake on plasma proteins measured by the Proseek Multiplex CVD II kit. This investigation showed that the plasma levels of 27 of the 90 investigated biomarkers were affected by food intake. The changes were modest in about half of the cases. Fourteen biomarkers were altered 10% or more 120 minutes after food intake. The observed effects of food intake for theses biomarkers are approximately equal or higher than the intra- and intraassay variation, which are about 10% for most of the biomarkers. There were, however, some exceptions. The biggest differences were observed for Heat shock 27 kDa protein, Proto-oncogene tyrosine-protein kinase Src, Growth hormone, Carbonic anhydrase 5A mitochondrial, Carcinoembryonic antigen related cell adhesion molecule 8, Fatty acid-binding protein intestinal, Pentraxin-related protein PTX3, Fibroblast growth factor 21, C-C motif chemokine 3, 2,4-dienoyl-CoA reductase mitochondrial, Gastrotropin, Poly [ADP-ribose] polymerase 1, Interleukin-6, and Melusin. The post food intake changes of 10-52% for these biomarkers suggest that not standardizing food intake prior to sampling can introduce additional variation. It is possible to measure multiple plasma proteins simultaneously with for example the Proseek Multiplex CVD II kit and this technology has recently been used in a multitude of studies (Edsfeldt et al., 2016; Goncalves et al., 2015; Gonçalves et al., 2016; Lind et al., 2015a; Lind et al., 2015b; Wiklund et al., 2016; Schiopu et al., 2016), and our investigation adds methodological information to this developing field. We have previously reported the effects of food intake on biomarkers analyzed with the Proseek Multiplex CVD III and Proseek Multiplex Neurology I kits (Dencker M et al., 2017a; Dencker M et al., 2017b), and the findings for those kits were rather different than the Proseek Multiplex CVD II kit. In the Proseek Multiplex CVD III only 17 biomarkers were affected by food intake, and only six biomarkers showed a difference of 10% or more due to food intake (Dencker M et al., 2017b). The effect of food intake was even smaller with the Proseek Multiplex Neurology I kit, out of 92 neurological biomarkers only 14 were significantly affected by food intake, and only one biomarker showed a difference over 10% due to food intake (Dencker M et al., 2017a).

We are aware of only one study that has evaluated the impact of food intake on the biomarkers in the present study on a large scale. Jahn and co-workers evaluated the effect of food intake on 34 of the 90 biomarkers in the present study (Jahn et al., 2016). This study evaluated the biomarker levels before and 240 minutes after a high-fat breakfast in 16 healthy and 18 metabolic syndrome subjects. Their findings were partly at odds with the present investigation. The findings in the study by Jahn et al. were concordant for 18 biomarkers (Placenta growth factor, Interleukin-1 receptor antagonist protein, Angiopoietin-1 receptor, Tissue factor, Interleukin-27, TNF-related apoptosis-inducing ligand receptor 2, Fibroblast growth factor 23, Stem cell factor, Interleukin-18, Renin, Pro-interleukin-16, Receptor for advanced glycosylation end products, Dickkopf-related protein 1, Agouti-related protein, Proheparin-binding EGF-like growth factor, Matrix metalloproteinase-12, Cathepsin L1, and Follistatin). The findings, however, were discordant for 16 biomarkers (CD40 ligand, Proto-oncogene tyrosine-protein kinase Src, Interleukin-6, Proteinase-activated receptor 1, Platelet-derived growth factor subunit B, C-X-C motif chemokine 1, Lectin-like oxidized LDL receptor 1, Heat shock 27 kDa protein, Growth hormone, Pappalysin-1, Pentraxin-related protein PTX3, P-selectin glycoprotein ligand 1, C-C motif chemokine 3, Matrix metalloproteinase-7, Vascular endothelial growth factor D, and Leptin). The differences can most probably be explained by the differences in statistical analysis, observation time, or the differences in the meal composition between the studies. These differences highlight the need for more comprehensive studies in the future. There are some limitations with this study that should be addressed. The present investigation studied the effect of food intake only in young healthy Caucasian subjects. Studies are warranted in older healthy subjects, different ethnic groups, and in patients with various cardiac diseases to determine whether the findings in the present investigation are reproducible in such populations. Future investigations should also address the effect of meal size and different diets such as high or low fat (McFarlin et al., 2017).

CONCLUSION

The present investigation shows that 27 biomarkers for CVD and inflammation measured by the Proseek Multiplex CVD II kit are affected by food intake, but the effect is modest in about half of the cases. There are, however, some exceptions. Caution concerning food intake should be taken when investigating Heat shock 27 kDa protein, Proto-oncogene tyrosine-protein kinase Src, Growth hormone, Carbonic anhydrase 5A mitochondrial, Carcinoembryonic antigen related cell adhesion molecule 8, Fatty acid-binding protein, intestinal, Pentraxin-related protein PTX3, Fibroblast growth factor 21, C-C motif chemokine 3, 2,4-dienoyl-CoA reductase mitochondrial, Gastrotropin, Poly [ADP-ribose] polymerase 1, Interleukin-6, and Melusin.

COMPETING INTERESTS

None.

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